

X-ray Structure Determination of Human Profilin II: a Comparative Structural Analysis of the Two Human Profilins

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Introduction: Profilin regulates actin filament assembly, and like SH3 domains also directly binds to proline-rich stretches on a number of cytoskeletal proteins. We have determined the crystal structure of human profilin II, the brain-enriched isoform, and performed a comparative structural analysis of the human isoforms.

Methods and Materials: Mosaic crystals of recombinant human profilin II were obtained in 1.35M magnesium sulfate concentrations, 1.5% (v/v) PEG 400, pH 7.3. Collecting the data on relatively small (<0.1mm. for all dimensions), less defective crystals at the synchrotron was critical to obtaining a high-resolution dataset, which was not possible with an in-house generator.

Results: An aromatic extension in the poly-L-proline binding site is evident in profilin II, with most of the charged differences proximal to the poly-L-proline binding site. The actin-binding face has remained highly conserved with the exception of five positions, most of which lie on the periphery of the actin binding site.

Conclusions: The aromatic extension and charged differences lining the poly-L-proline binding site may play a role in the differential affinities displayed by the two mammalian profilin isoforms for proline-rich stretches in various protein targets. These studies suggest the construction of mutants, which will aid in understanding the specificity of the two profilin isoforms for proline-rich ligands.

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